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Tardigrada and Nematoda associations with lichen and bryophyte habitats from Southwest Wisconsin state parks, universities, and private land

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SUMMARY

Microscopic terrestrial invertebrates, such as tardigrades and nematodes, have been historically understudied and misunderstood. Terrestrial habitats of tardigrades and nematodes, like moss and lichens, have been sparingly identified throughout scientific and natural history. Additionally, many regions within the United States have little-to-no records of tardigrade and nematode taxa from moss and lichens. In the current study, we collected moss, lichen, and liverwort samples from multiple state parks and areas within Southwest Wisconsin to investigate tardigrade and nematode communities. Generalized Linear Models confirmed that habitat species, substrate, site location, and elevation had a significant effect on tardigrade and nematode abundances. We also report significant associations of certain tardigrade and nematode taxa with their respective cryptogam habitats. Furthermore, we report a new distribution of the heterotardigrade species *Viridiscus* aff. *perviridis* and *Viridiscus viridissimus*, and the eutardigrade species *Minibiotus* cf. *jonesorum* to the state of Wisconsin. Our study indicates that many tardigrade and nematode associations with cryptogam habitats have yet to be explored and documented in North America.

INTRODUCTION

Tardigrades are microscopic, eight-legged invertebrates that are known for their cryptobiotic abilities that allow them to survive harsh conditions like desiccation, freezing temperatures, high levels of radiation, extreme pressures, and even the conditions of outer space at Low Earth Orbit (Miller 1997, Jönsson et al. 2008). Although tardigrades are becoming increasingly popular among scientists, ecologists, and taxonomists, they have historically been understudied animals (Nelson et al. 2018). There are many unknowns in the fields of tardigrade taxonomy and ecology. For instance, there are 1,488 species of tardigrades known to science (Degma and Guidetti 2024), however, when compared to 28,537 species of nematodes (Hodda 2022), it is clear there are likely hundreds to thousands of tardigrade species yet to be discovered and documented. Nematodes are microscopic roundworms that are also known for their tolerance of harsh environmental pressures, such as heating and freezing conditions (Adhikari et al. 2010, Mukuka et al. 2010). Some nematode species are commonly known as agricultural pests, and knowing which species are present in certain environments is important for farmers and scientists to determine soil health (MacGuidwin 2018).

Both tardigrades and nematodes have been commonly found to inhabit moss, lichens, leaf litter and soils (Sánchez-Moreno et al. 2006, Kagoshima et al. 2012, Guil et al. 2015, Collins and Goudie 2020, Ankrom et al. 2022, Kitagami and Matsuda 2023). Annotated Zoogeography reports of non-marine tardigrades in multiple countries and continents, such as North America, report that many past studies have not identified the moss or lichen species containing the tardigrade species they documented (Kaczmarek et al. 2016, McInnes et al. 2017, Michalczyk et al. 2022). While some studies have investigated nematode populations in moss and lichens (Barbuto and Zullini 2006, Bokhorst et al. 2015, Shevchenko and Zhylina 2021), many of these studies do not identify the species of moss and lichen. Studies looking at nematodes in moss and lichens have also been sparingly conducted compared to studies investigating nematodes in soils (Sánchez-Moreno et al. 2006, Darby et al. 2007, MacGuidwin, 2018). Essentially, tardigrade and nematode associations with their respective moss and lichen habitats remains a mystery in many areas of the world.

In the United States, some states still have no records of any tardigrade taxa (Kaczmarek et al. 2016, Cotten and Miller 2022). In the state of Wisconsin, only 13 species of tardigrades have been documented from a total of five reports (Mathews 1938, Ramazzotti 1956, Ramazzotti 1957, Ramazzotti and Maucci 1983, Maucci 1987) with the first report being 87 years ago and the last report being 38 years ago. Throughout these five reports of tardigrade species from Wisconsin, there was no mention of the moss or lichen taxa. We are left to conclude that there is no current knowledge of the associations of tardigrades and their specific habitats in Wisconsin. In contrast, nematode associations with habitats and crops have been documented in Wisconsin in multiple studies (MacGuidwin 2018, Ye et al. 2018, Han et al. 2022). Many have also been reports of parasitic nematodes to many organisms, such as Diptera larvae (Anderson and DeFoliart 1962), fish (Amin 1984), ermine (Dubay et al. 2014), soybeans (Saikai et al. 2019, MacGuidwin et al. 2023), corn and potatoes (Dickerson et al. 1964), and grass (Norton et al. 1987). Nematode taxa in moss from Wisconsin has been sparingly documented (Thorne 1924).

Tardigrade taxonomic classifications are currently undergoing drastic changes with new genera and species being added to the species checklist within the last decade (Gąsiorek et al. 2019, Gąsiorek et al. 2019, Stec et al. 2021, Zawierucha et al. 2023, Degma and Guidetti 2024). Depending on the tardigrade taxa and microscope resolution, order to genus identification is possible with a dissecting microscope, and genus to species identification is possible with a compound microscope. However, in many cases, morphometrics, morphological identification of eggs, phase contrast and SEM images, or genetic sequences are recommended for species identifications (Nelson et al. 2020, Morek et al. 2021). When considering the changes in tardigrade taxonomy and difficulty in identifying species, it is clear that past records and documentation may not be sufficient for knowing the distribution of tardigrade species as they are currently known.

The aim of the study was to explore the tardigrade, nematode and cryptogam associations of Southwest Wisconsin. The main hypothesis was that habitat species (e.g., moss, lichen and liverwort species), substrate (e.g., tree base, tree branch, tree trunk, rock, soil), site location, and elevation would have a significant effect on tardigrade and nematode abundance. Another hypothesis was that there would be significant associations between tardigrade genera and the habitat species they reside in. We also hypothesized that we would find significant associations between nematode genera and the habitat species they inhabit. Additionally, given the low records of tardigrade species in Wisconsin, our last hypothesis was that we would find evidence of new distribution of tardigrade species to the state.

MATERIALS AND METHODS

Sample collections

Lichen and moss samples were randomly collected between March 2021 and October 2022 from multiple areas within Southwest Wisconsin, such as Devil's Lake State Park, Governor Dodge State Park, Belmont Mound State Park, Wyalusing State Park, the University of Wisconsin-Madison arboretum, the University of Wisconsin-Platteville campus, and private land (Fig. 1). Liverwort samples were collected from Wyalusing State Park. Samples were collected into paper envelopes to facilitate desiccation preservation for storage, and marked with a unique sample code. In total, 74 lichen, 10 liverwort, and 33 moss samples (N=117) were obtained from Southwest Wisconsin for the extraction of tardigrades and nematodes. Coordinates, aspect, elevation and pictures of samples were taken with an iPhone X.

Sample processing

After collection, lichen, moss and liverwort specimens were examined using an Olympus SZX16 dissecting microscope and an Olympus BX51 compound microscope at the University of Colorado-Boulder Herbarium (COLO) where they could be compared to a robust cryptogam reference collection. Microscopic structures such as ascospores of lichens or leaf morphology of mosses were studied using hand-prepared sections cut using a razor blade then mounted on slides in water, iodine, or potassium hydroxide (the latter two being primarily for lichens). Chemistry, often needed for lichen species identification, was studied using standard spot tests (K, C, KC, P, UV) and procedures following Brodo et al. (2001). All taxonomic names of our lichen specimens were standardized based on Esslinger's latest North American lichen checklist (2021) and the Consortium of Lichen Herbaria (2023) and taxonomic names of our moss specimens were standardized using Consortium of Bryophyte Herbaria (2023). Lichen identification was based on numerous keys written by regional specialists including Thomson (2003), Brodo (2016), and McMullin (2023). Moss and liverwort identification followed suit but relied on Crum (1973), Crum and Anderson (1981), and Klips (2022) as well as keys provided on the online Flora of North America (2007, 2014).

A subsample of each lichen, moss and liverwort sample was crushed up and weighed out in a plastic weigh boat to 0.500 gram on a Mettler Toledo PL303 scale. Subsamples were placed in a Baermann funnel with a Kimtech wipe as the filter for tardigrade and nematode extractions. Three separate 20mL extractions (i.e., one extraction each day for three days) were taken and combined into one 50mL conical tube for each subsample. After the second extraction and before the third, the volume was reduced to accommodate the volume of the third extraction.

After the third extraction, the conical tube was left to sit for one hour to let tardigrades and nematodes sink to the bottom of the tube. Once extractions were ready to be processed, the volume in the tube was reduced to 5mL with a vacuum pump and poured into a 35 x 10mm gridded petri dish (Ankrom et al. 2022).

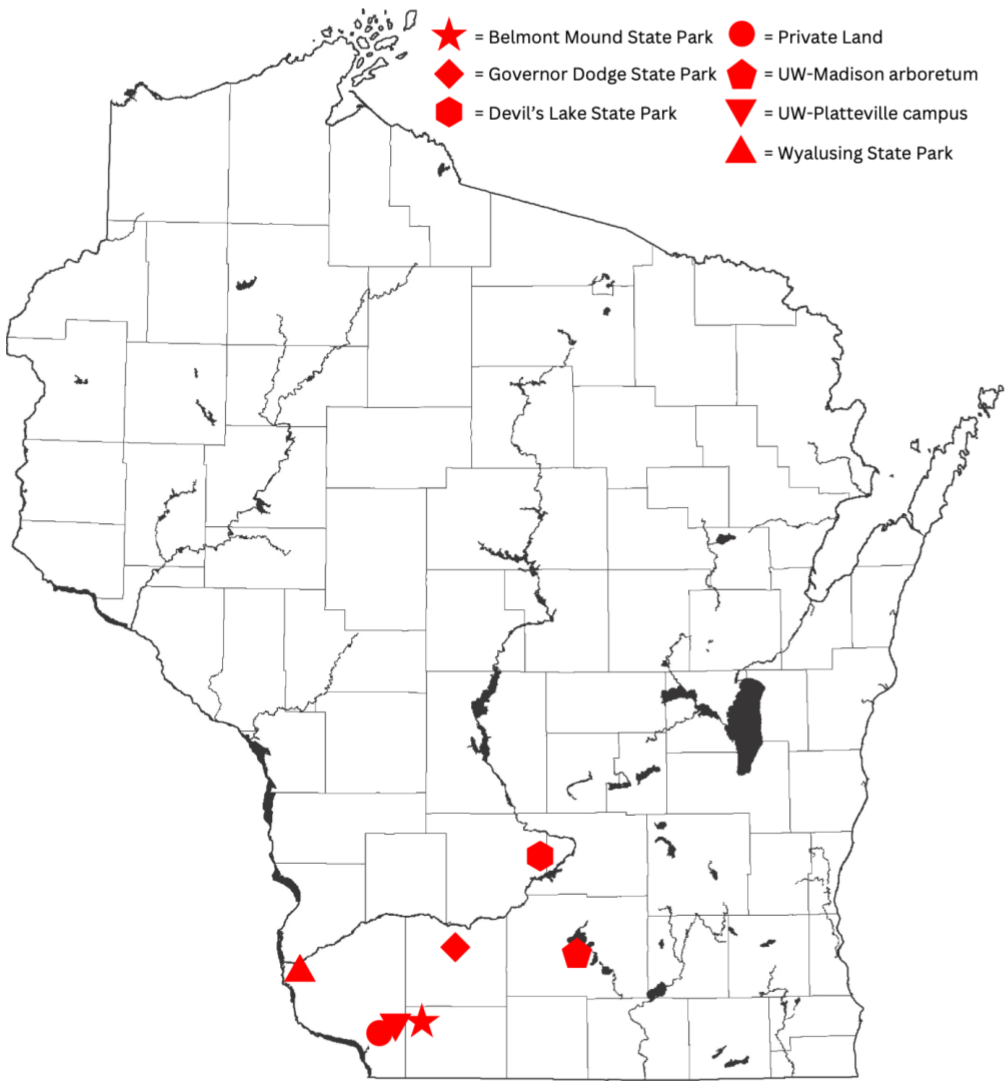

Figure 1. Map of collection sites in Southwest Wisconsin.

Tardigrade and nematode abundance counts were conducted using an Olympus CKX41 inverted compound microscope at 200X-400X to scan through the entirety of the gridded petri dishes. Afterwards, using an Olympus SZX12 dissecting microscope, voucher specimens and wet mounts were made of every tardigrade and nematode population noted in the abundance counts. Wet mounts were made of at least 10% or the first 100 individuals of each tardigrade and nematode population (Darby et al. 2007). Three to five permanent voucher specimens were made from each population. Each slide was labeled with the corresponding unique sample code and a taxon identification. Voucher specimens were collected by fishing individual animals out of the petri dish with an Irwin loop, placing them in CMCP-10 mounting media on microscope slides, and covering the mounting media with a cover slip (Miller 1997).

Tardigrade and nematode identification was conducted utilizing voucher specimens and wet mounts for phase contrast compound microscopy at 400X and 1,000X with an Olympus BX41 compound microscope. Furthermore, tardigrade and nematode family to species identifications were possible using morphological keys and world literature (Ramazzotti and Maucci 1983, Bongers 1988, Pilato and Binda 2010, Gąsiorek et al. 2019, Stec et al. 2020, Stec et al. 2021, Hodda 2022, Degma and Guidetti 2024). Specimens were imaged using an Olympus BX41 compound microscope with phase contrast and darkfield settings or an Olympus BX60 compound microscope equipped with DIC (Differential Interference Contrast). Voucher specimens from this study were donated to collections at the University of Wisconsin-Platteville and the University of Wisconsin-Madison's arboretum.

Statistical analyses

Generalized Linear Models with Poisson regression accompanied by a One-Way ANOVA were used to investigate any significant effects that habitat species, substrate, site location, and elevation had on tardigrade and nematode abundance. Tukey's Post Hocs were used to compare means of tardigrade and nematode genera abundance among habitat species when $N \geq 3$. Only p-values with a value of < 0.05 were considered significant. Biostatistics and data visualization were conducted using RStudio (RStudio Team 2020). R codes used in this study are provided (Supplementary Material 1).

RESULTS

Sample and specimen collections

In total, one species of liverwort, 11 species of moss, and 17 species of lichen were identified from the samples collected from Southwest Wisconsin (Figs. 2 & 3) (Table 1). Overall, we found 2,981 tardigrades and 11,533 nematodes among the 117 samples collected. Tardigrade genera collected include *Milnesium* Doyère, 1840, *Ramazzottius* Binda & Pilato, 1986, *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009, *Macrobiotus* C.A.S. Schultze, 1834, *Viridiscus* Gąsiorek & Michalczyk, 2019, *Claxtonia* Gąsiorek & Michalczyk 2019, *Pseudechiniscus* Thulin, 1911, *Minibiotus* R.O. Schuster, 1980, *Diphascon* Plate, 1888, and *Hypsibius* Ehrenberg, 1848. Nematode genera collected included all main nematode trophic groups: the bacterivores *Plectus* Bastian, 1865, *Eumonhystera* Andrassy, 1981, *Cervidellus* Thorne, 1937, *Teratocephalus* de Man, 1876, *Wilsonema* (de Man, 1880) Cobb, 1913, and *Heterocephalobus* Brzeski, 1961, the fungivores *Aphelenchus* Bastian, 1865 and *Aphelenchoides* Fischer, 1894, the herbivores *Tylenchus* Bastian, 1865 and *Tylenchorhynchus* Cobb, 1913, the omnivore *Prodorylaimus* Andrassy, 1959, and the predators *Tripyla* Bastian, 1865 and *Clarkus* Jairajpuri, 1970. We also found other nematode taxa identified as Dorylaimidae, and a few unknown (Table 1) (Supplementary Table S1).

In the current study, we found morphological evidence of the presence of the genus *Viridiscus* in Wisconsin. This is the first occurrence of the genus *Viridiscus* in Wisconsin (Kaczmarek et al. 2016, Miller and Perry 2019). We also report finding the species *Viridiscus* aff. *perviridis* (Ramazzotti, 1959) (Fig. 4) and *Viridiscus viridissimus* (Péterfi, 1956) (Fig. 5) at Belmont Mound State Park, and *Minibiotus* cf. *jonesorum* Meyer, Lyons, Nelson, & Hinton, 2011 (Fig. 6) at Devil's Lake State Park which are the first records of these species in the state of Wisconsin (Kaczmarek et al. 2016, Miller and Perry 2019). The specific locations, habitat species and types of substrate for these records are included in Table 2.

Table 1. Sample size (number of samples per species), moss/lichen/liverwort species name (habitat species), type of organism (habitat group), growth form, and tardigrade and nematode taxa found in each species.

N	Habitat Species	Habitat Group	Growth Form	Tardigrade Taxa	Nematode Taxa
3	*Canoparmelia texana* (Tuck.) Elix & Hale	Lichen	Broad-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*	*Plectus*, *Eumonhystera*
3	*Dermatocarpon miniatum* (L.) W. Mann	Lichen	Umbilicate Foliose	*Milnesium*	*Plectus*, *Eumonhystera*
5	*Flavoparmelia baltimorensis* (Gyelnik & Fóriss) Hale	Lichen	Broad-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*, *Minibiotus*, *Pseudechiniscus*	*Plectus*, *Eumonhystera*, *Clarkus*, *Cervidellus*
1	*Flavoparmelia caperata* (L.) Hale	Lichen	Broad-lobed Foliose	No tardigrades found	*Plectus*
2	*Parmotrema stuppeum* (Taylor) Hale	Lichen	Broad-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Claxtonia*	*Plectus*, *Eumonhystera*
6	*Phaeophyscia adiastola* (Essl.) Essl.	Lichen	Narrow-lobed Foliose	*Milnesium*, *Macrobiotus*, *Paramacrobiotus*, *Viridiscus*	*Plectus*, *Eumonhystera*, *Clarkus*, *Aphelenchoides*, *Teratocephalus*, *Prodorylaimus*, *Wilsonema*
1	*Phaeophyscia ciliata* (Hoffm.) Moberg	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*	*Plectus*, *Eumonhystera*
12	*Physcia aipolia* (Ehrh. ex Humb.) Fürnr.	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*, *Claxtonia*	*Plectus*, *Eumonhystera*, *Clarkus*, *Aphelenchoides*, Dorylaimidae, Unknown
5	*Physcia halei* J. W. Thomson	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*	*Plectus*, *Eumonhystera*
1	*Physcia millegrana* Degel.	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*	*Plectus*, *Eumonhystera*
6	*Punctelia bolliana* (Müll. Arg.) Krog	Lichen	Broad-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*, *Claxtonia*	*Plectus*, *Eumonhystera*, *Clarkus*, Dorylaimidae
2	*Punctelia missouriensis* G. Wilh. & Ladd	Lichen	Broad-lobed Foliose	*Macrobiotus*, *Claxtonia*, *Minibiotus*	*Plectus*, *Eumonhystera*, *Clarkus*, *Aphelenchoides*, Dorylaimidae
3	*Punctelia rudecta* (Ach.) Krog	Lichen	Broad-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*	*Plectus*, *Eumonhystera*, *Aphelenchoides*
10	*Rusavskia elegans* (Link) S. Y. Kondr. & Kärnefelt	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*	*Plectus*, *Eumonhystera*, *Aphelenchus*
10	*Xanthomendoza fallax* (Hepp ex Arnold) Søchting, Kärnefelt & S. Y. Kondr.	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Macrobiotus*, *Claxtonia*	*Plectus*, *Eumonhystera*, *Aphelenchoides*
1	*Xanthomendoza ulophyllodes* (Räsänen) Søchting, Kärnefelt & S. Y. Kondr.	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Macrobiotus*, *Viridiscus*	*Plectus*, *Eumonhystera*, *Prodorylaimus*, Dorylaimidae
3	*Xanthomendoza weberi* (S. Y. Kondr. & Kärnefelt) L. Lindblom	Lichen	Narrow-lobed Foliose	*Milnesium*, *Ramazzottius*, *Paramacrobiotus*, *Claxtonia*	*Plectus*, *Eumonhystera*, *Aphelenchus*

10	*Conocephalum salebrosum* Szweyk., Buczk. & Odrzyk.	Liverwort	Complex thalloid	*Ramazzottius*	*Eumonhystera*, *Aphelenchoides*, *Tylenchus*, *Heterocephalobus*, *Tylenchorhynchus*
7	*Anomodon attenuatus* (Hedw.) Ignatov & Fedosov	Moss	Pleurocarp	*Ramazzottius*, *Macrobiotus*	*Plectus*, *Clarkus*, *Aphelenchus*, *Aphelenchoides*, *Tripyla*, *Teratocephalus*, *Prodorylaimus*, Dorylaimidae, Unknown
2	*Brachythecium falcatum* (Grout) H.A. Crum	Moss	Pleurocarp	*Paramacrobiotus*	*Plectus*, *Clarkus*
7	*Brachythecium laetum* (Brid.) Schimp.	Moss	Pleurocarp	*Milnesium*, *Ramazzottius*, *Pseudechiniscus*, *Minibiotus*, *Hypsibius*	*Plectus*, *Aphelenchus*, *Aphelenchoides*, *Tripyla*, *Prodorylaimus*, *Wilsonema*
5	*Bryoandersonia illecebra* (Hedw.) H. Rob.	Moss	Pleurocarp	*Milnesium*, *Ramazzottius*	*Plectus*, *Wilsonema*
1	*Claopodium rostratum* (Hedw.) Ignatov	Moss	Pleurocarp	*Ramazzottius*	*Plectus*, *Prodorylaimus*
1	*Dicranum spurium* Hedw.	Moss	Acrocarp	No tardigrades found	*Teratocephalus*, *Prodorylaimus*
1	*Grimmia plagiopodia* Hedw.	Moss	Acrocarp	No tardigrades found	No nematodes found
2	*Orthodicranum montanum* (Hedw.) Loeske	Moss	Acrocarp	*Milnesium*, *Macrobiotus*, *Minibiotus*	*Plectus*, *Aphelenchoides*, *Teratocephalus*, *Prodorylaimus*, *Wilsonema*, Dorylaimidae, Unknown
5	*Orthotrichum anomalum* Hedw.	Moss	Acrocarp	*Milnesium*, *Ramazzottius*, *Macrobiotus*, *Viridiscus*	*Plectus*, *Aphelenchoides*, *Prodorylaimus*
1	*Schistidium crassithecium* H.H. Blom ex B.H. Allen	Moss	Acrocarp	No tardigrades found	No nematodes found
1	*Thuidium delicatulum* (Hedw.) Schimp.	Moss	Pleurocarp	*Ramazzottius*, *Diphascon*	*Plectus*, *Eumonhystera*, *Prodorylaimus*

Table 2. Biogeography and ecology information for new distribution records of tardigrade species.

Species	**City (State)**	**Coordinates**	**Habitat Species**	**Substrate**
Minibiotus cf. *jonesorum*	Baraboo (WI)	43.426944, -89.726111; 43.425, -89.724722; 43.425278, -89.724167	*Brachythecium laetum* (moss); *Flavoparmelia baltimorensis* (lichen); *Orthodicranum montanum* (moss)	Rock (Quartzite)
Viridiscus aff. *perviridis*	Belmont (WI)	42.770833, -90.348056	*Phaeophyscia adiastola* (lichen)	Rock (Limestone)
Viridiscus viridissimus	Belmont (WI)	42.770278, -90.348333	*Xanthomendoza ulophyllodes* (lichen)	Rock (Limestone)

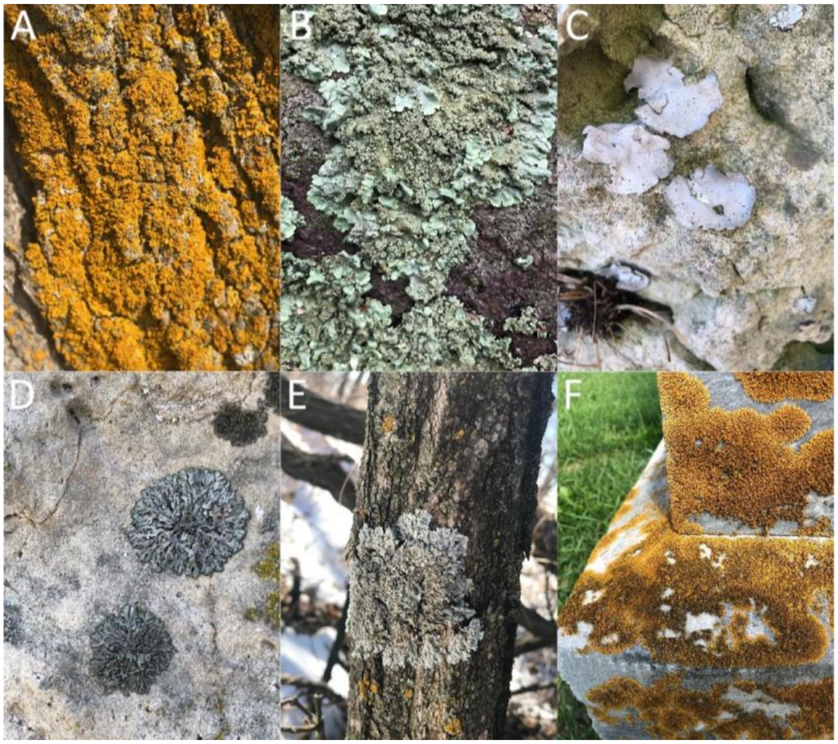

Figure 2. Lichens sampled in this study. A *Xanthomendoza fallax*, B *Flavoparmelia baltimorensis*, C *Dermatocarpon miniatum*, D *Phaeophyscia adiastola*, E *Physcia aipolia*, F *Rusavskia elegans*.

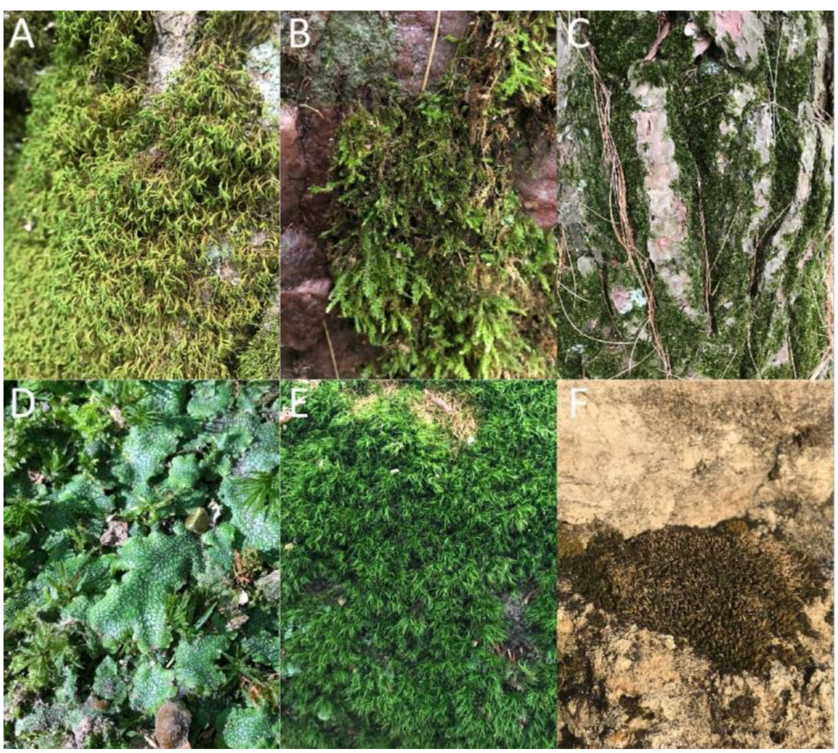

Figure 3. Bryophytes sampled in this study. A *Anomodon attenuatus*, B *Brachythecium laetum*, C *Bryoandersonia illecebra*, D *Conocephalum salebrosum*, E *Orthodicranum montanum*, F *Orthotrichum anomalum*.

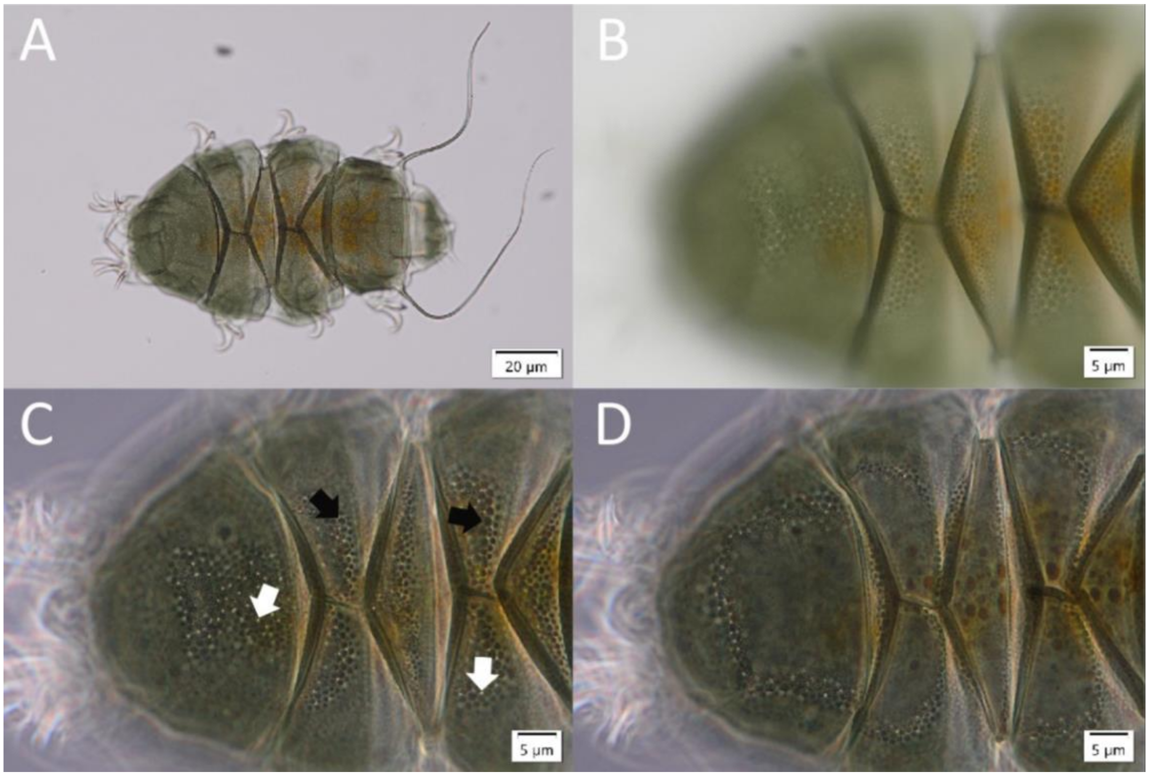

Figure 4. Compound micrographs of *Viridiscus* aff. *perviridis* from Belmont Mound State Park (Belmont, WI). A Transmitted light at 400X, B Transmitted light at 1,000X, C Phase contrast at 1,000X (black arrows = granulation, white arrows = light spots or pores), D Phase contrast at 1,000X. Scale bar for A is 20 micrometers. Scale bars for B - D are 5 micrometers.

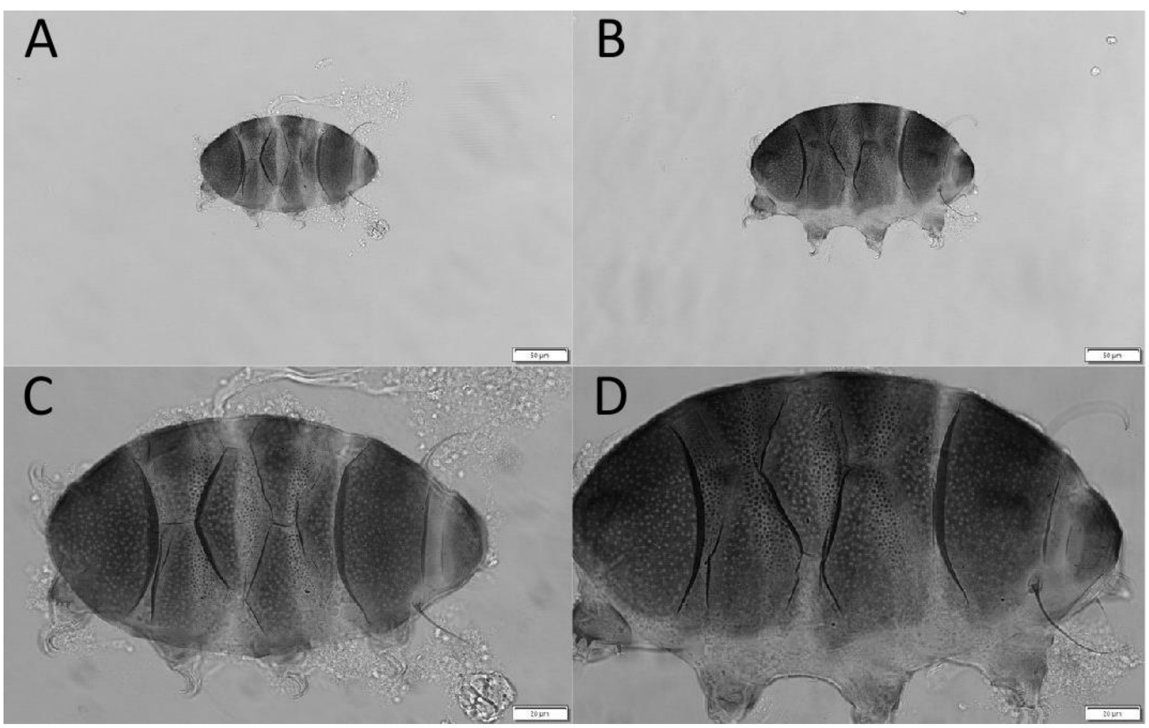

Figure 5. Compound micrographs of two *Viridiscus viridissimus* specimens from Belmont Mound State Park (Belmont, WI) A Transmitted light at 400X, B Transmitted light at 400X, C Transmitted light at 1,000X, D Transmitted light at 1,000X. Scale bars for A - B are 50 micrometers. Scale bars for C - D are 20 micrometers.

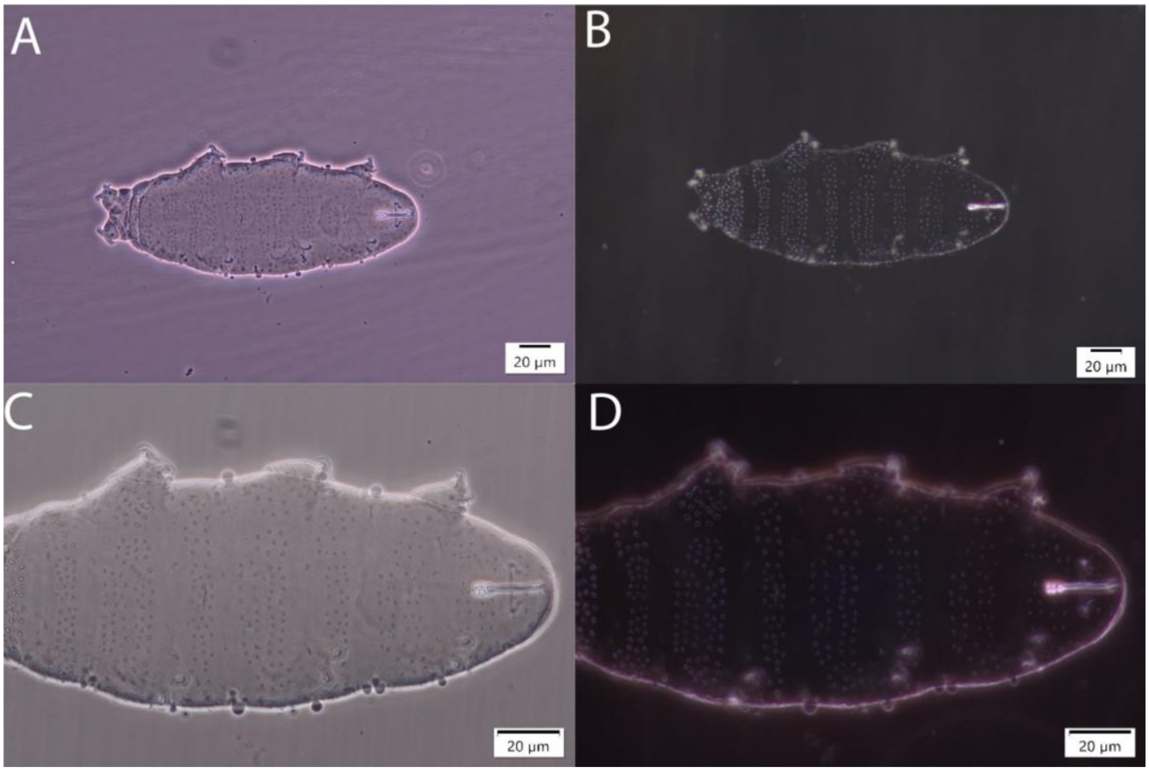

Figure 6. Compound micrographs of *Minibiotus* cf. *jonesorum* at 200X and 400X from Devil's Lake State Park (Baraboo, WI). A Phase contrast at 200X, B Darkfield at 200X, C Phase contrast at 400X, D Darkfield at 400X. Scale bars are 20 micrometers.

Tardigrade and nematode abundance across habitats and substrates

Conducting Generalized Linear Models with Poisson regression followed by One-Way ANOVAs showed habitat species, substrate, site location, and elevation as having significant effects on tardigrade and nematode abundances in Southwest Wisconsin (Table 3). Relative abundances of tardigrades among their respective habitat species, substrates, and site locations are shown in Figure 7. Relative abundances of nematodes among their respective habitat species, substrates, and site locations are shown in Figure 8.

The Tukey's Post Hoc results for tardigrades in habitat species revealed that *Milnesium* were significantly more abundant in *Physcia halei* compared to *Physcia aipolia*, *Brachythecium laetum*, *Conocephalum salebrosum*, and *Anomodon attenuatus*. *Ramazzottius* was significantly more abundant in *Xanthomendoza fallax* compared to *C. salebrosum*. *Viridiscus* was significantly more abundant in *Phaeophyscia adiastola* compared to *Xanthomendoza weberi*, *X. fallax*, *Punctelia bolliana*, *Punctelia rudecta*, *Rusavskia elegans*, *P. aipolia*, *P. halei*, *Parmotrema stuppeum*, *Orthotrichum anomalum*, *Flavoparmelia baltimorensis*, *Dermatocarpon miniatum*, *C. salebrosum*, *Canoparmelia texana*, *Bryoandersonia illecebra*, *B. laetum*, and *Anomodon attenuatus*. *Minibiotus* was significantly more abundant in *F. baltimorensis* compared to *X. fallax*, *P. bolliana*, *R. elegans*, *P. aipolia*, *P. halei*, *P. adiastola*, *O. anomalum*, *C. salebrosum*, *B. illecebra*, *B. laetum*, and *A. attenuatus* (see Supplementary Table S2).

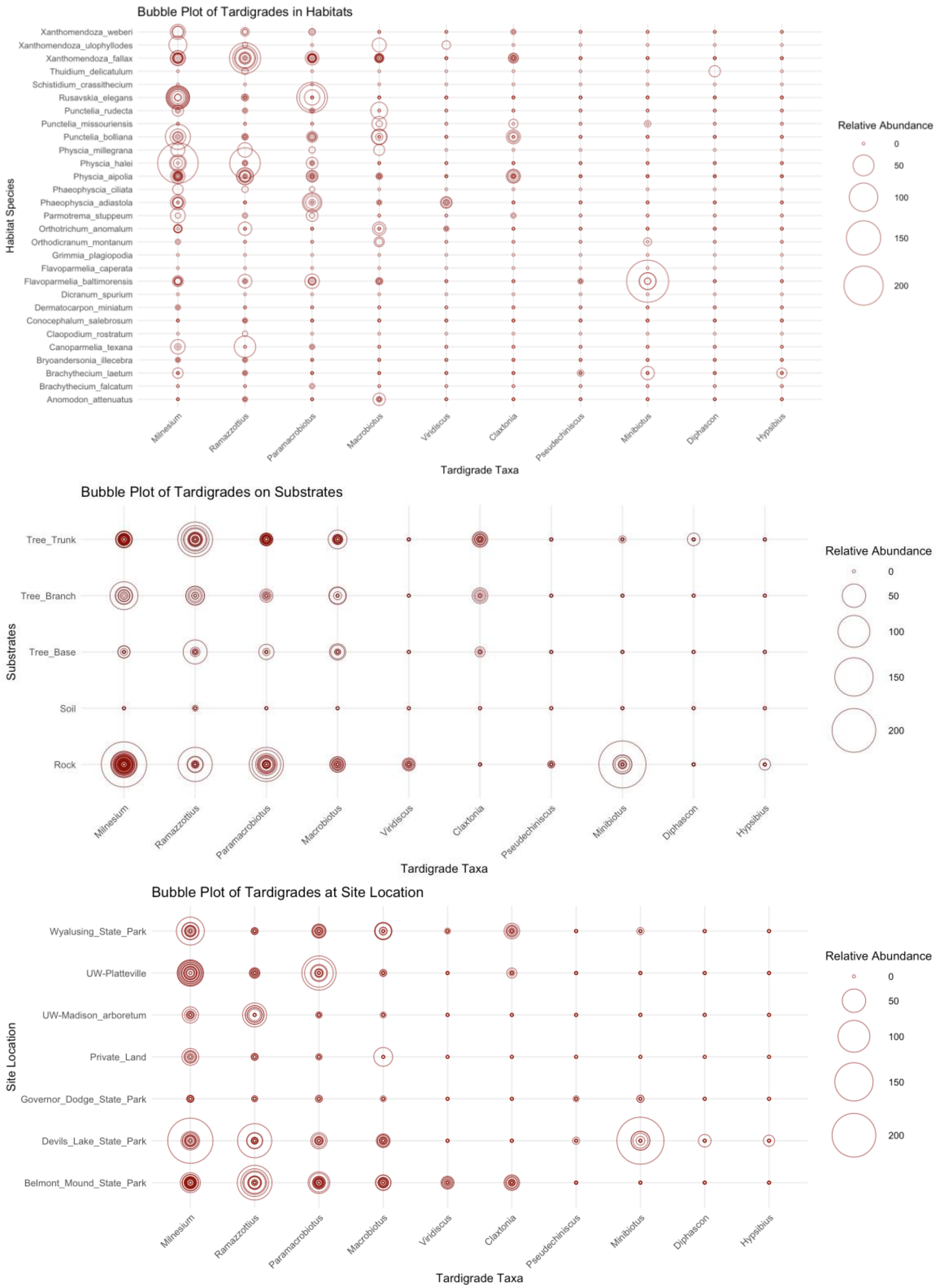

Figure 7. Bubble plots of relative abundance of tardigrade genera in habitat species, substrates, and site locations.

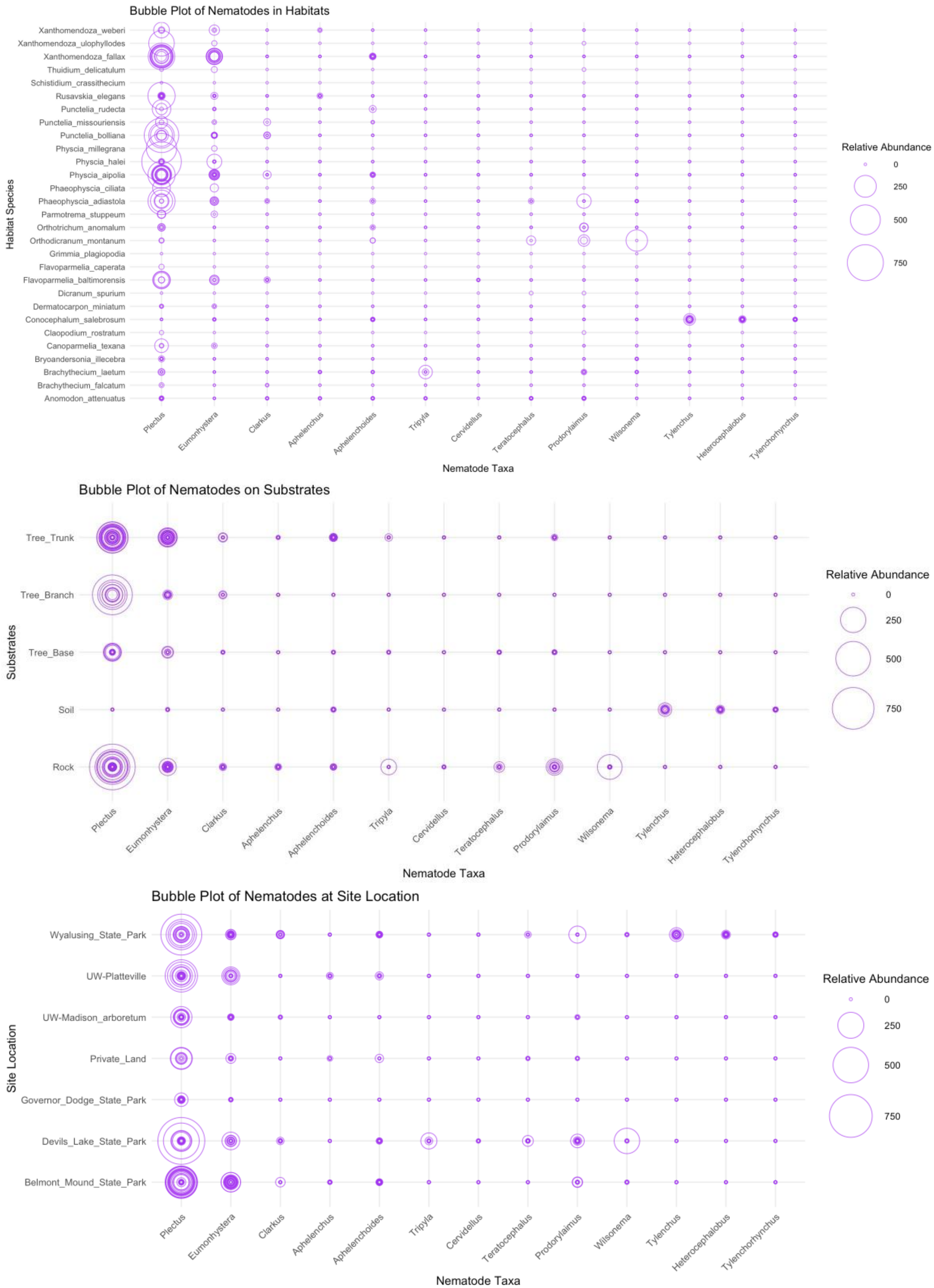

Figure 8. Bubble plots of relative abundance of nematode genera in habitat species, substrates, and site locations.

Table 3. The results of the Generalized Linear Models with Poisson regression followed by a One-Way ANOVA. P-values in bold are significant ($p < 0.05$).

Organisms	Variable	Pr(>Chisq)
Tardigrades	Habitat Species	**<2.2e-16**
	Substrate	**<2.2e-16**
	Site Location	**<2.2e-16**
	Elevation (m)	**0.002856**
Nematodes	Habitat Species	**<2.2e-16**
	Substrate	**<2.2e-16**
	Site Location	**<2.2e-16**
	Elevation (m)	**7.663e-09**

The Tukey's Post Hoc results for nematodes in habitat species revealed that *Eumonhystera* was significantly more abundant in *X. fallax* compared to *X. weberi*, *P. rudecta*, *P. bolliana*, *P. halei*, *P. aipolia*, *P. adiastola*, *P. stuppeum*, *O. anomalum*, *F. baltimorensis*, *D. miniatum*, *C. texana*, *B. illecebra*, and *A. attenuatus*. *Cervidellus* was significantly more abundant in *F. baltimorensis* compared to *P. aipolia*. *Tylenchus* and *Heterocephalobus* were significantly more abundant in *C. salebrosum* compared to *X. weberi*, *X. fallax*, *P. rudecta*, *R. elegans*, *P. bolliana*, *P. halei*, *P. aipolia*, *P. adiastola*, *O. anomalum*, *F. baltimorensis*, *D. miniatum*, *C. texana*, *B. illecebra*, *B. laetum*, and *A. attenuatus*. *Tylenchorhynchus* was significantly more abundant in *C. salebrosum* compared to *X. fallax*, *R. elegans*, *P. bolliana*, *P. aipolia*, *P. adiastola*, *B. laetum*, *A. attenuatus* (see Supplementary Table S2).

DISCUSSION

In the current study, certain nematode and tardigrade taxa showed significantly different abundances among habitat species. The results of our Generalized Linear Models suggest that habitat species and substrates had a significant effect on tardigrade and nematode abundance. Substrates and habitats have been previously reported to influence nematode and tardigrade abundance in other areas of the world (Sohlenius et al. 2004, Kaczmarek et al. 2011, Raymond et al. 2013, Bokhorst et al. 2015, Nelson et al. 2020).

Although terrestrial nematodes are commonly considered soil inhabitants, previous research showed that more than 200 nematode species inhabit European mosses, including bacterivores, fungivores, herbivores, omnivores, and predators (Barbuto and Zullini 2006). Our results report a low number of nematode genera associated with mosses and lichens in Wisconsin (13 genera) compared to previous records in other temperate forests. In sub-Mediterranean climates, 42 nematode genera were found associated with mosses in an India national park (Nesar et al. 2021), while 32 genera were found in Ukraine mosses (Shevchenko and Zhylina 2021). Previous research on nematodes associated with lichens in temperate areas is very scarce, although some lichen-inhabitant nematode species have been described (Vera et al. 2010).

In our study, *Plectus* was the most abundant and common genus of nematode, which is a similar pattern described by Sohlenius et al. (2004) and Barbuto and Zullini (2006). Shevchenko and Zhylina (2021) reported that Plectidae and Monhysteridae were the most abundant nematode families in *Orthotrichum* spp. mosses, and nematode genera such as *Aphelenchoides*, *Plectus*, and *Eumonhystera* were present. In our study, while *Plectus* (Plectidae) and *Eumonhystera* (Monhysteridae) were the most abundant nematodes overall, nematode populations found in *Orthotrichum anomalum* moss, the only *Orthotrichum* species sampled in the study, were composed of

Aphelenchoides, *Plectus*, and *Prodorylaimus*, and no *Eumonhystera* were present.

Although tardigrades and their respective lichen and moss habitats have been sparsely documented in the U.S., some associations have been documented (Kaczmarek et al. 2016). The nearest record to Wisconsin is from Iowa showing the species *Milnesium tardigradum* Doyére 1840 in the lichen species *Physcia millegrana* (Kimmel & Meglitsch 1969). Another close record to Wisconsin was in Kansas documenting *M. tardigradum* from the lichen species *Xanthoria* (=*Xanthomendoza*) *fallax* (Lehmann et al. 2007). In our study, we found *Milnesium* spp. on both *P. millegrana* and *X. fallax*, although none of our specimens matched the identity of *M. tardigradum*.

Our findings of *Viridiscus* aff. *perviridis*, *Viridiscus viridissimus*, and *Minibiotus* cf. *jonesorum* adds three new state records and brings the number to 16 species of tardigrades documented within Wisconsin's borders. The *V.* aff. *perviridis* specimens seemed to best fit the identity of *V. perviridis* based on the cirrus A length and granulation, although our specimens seem to potentially have light spots or pores, similar to that of *V. viridissimus*, which are sporadically placed between the granulation (Fig. 4). The discrepancies between these phenotypes indicate it is possibly a new species of *Viridiscus*. The original description of *M. jonesorum* states it has a white/translucent body (Meyer et al. 2011), whereas our specimens seemed to have a red/orange pigmentation consistent throughout the body. This brings into question whether *M. jonesorum* has variation in pigmentation or if our specimens may be representing a new species. Currently, no DNA barcode sequences are available as reference sequences for *M. jonesorum* identification (Altschul et al. 1990). Samples containing *V.* aff. *perviridis* and *M.* cf. *jonesorum* found in our study were sent to Jagiellonian University in Kraków, Poland for future genetic analyses and species comparison studies.

Overall, our results include a unique analysis of nematode and tardigrade associations with their respective lichen and bryophyte habitats. These associations have also not been thoroughly documented from surrounding states of Wisconsin, such as Minnesota, Iowa, and Illinois, that have similar ecosystems, weather, and temperatures. Additionally, there has been a lack of investigations into the geographical, topographical, and seasonal effects on tardigrade and nematode abundances in these areas. More studies in Northern Wisconsin and surrounding states are suggested to elucidate tardigrade-habitat and nematode-habitat associations in the Midwestern U.S. ecosystems of North America.

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SUPPLEMENTARY MATERIALS

Supplementary Material 1. R Code used in the current study.

Supplementary Table S1. Ecological data associated with samples featured in the current study.

Supplementary Table S2. Tukey's Post Hoc results of tardigrade and nematode genera in habitat species. Comparisons are in italics and p-values are bold.

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